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# Common flora and intestine

## A carcinogenic marriage

Kepeng Wang and Michael Karin*

Laboratory of Gene Regulation and Signal Transduction; Department of Pharmacology and Pathology; School of Medicine; University of California, San Diego; La Jolla, CA USA

**Commensal microflora engages in a symbiotic relationship with their host, and plays an important role in the development of colorectal cancer (CRC). Pathogenic bacteria promote chronic intestinal inflammation and accelerate tumorigenesis. In sporadic CRC, loss of an effective epithelial barrier occurs at early stage of CRC development. As a result, non-pathogenic bacteria and/or their products infiltrate tumor stroma, drive "tumor-elicited inflammation" and promote CRC progression by activating tumor-associated myeloid and immune cells that produce IL-23 and IL-17. In this article we will summarize the recent advances in understanding the relationship between gut flora and CRC.**

**Keywords:** commensal flora, colorectal cancer, inflammation, epithelial barrier, IL-23, IL-17

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*Correspondence to: Michael Karin;
Email: karinoffice@ucsd.edu

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Commensal bacteria reside on the epithelial surfaces of the gastro-intestinal tract, lung and skin and engage in a symbiotic relationship with their host.[1] Commensal microflora assist host metabolism, protect from infection by pathogenic bacteria and fungi, and serve as guides and educators for development of host immune system.[2-5] However, the seemingly happy marriage (symbiosis) between the commensal microflora and the host also carries the risk of household dispute. Dysregulated interaction between commensal and their host is a critical driving factor in the development of chronic inflammation, metabolic disorder, cardiovascular disease, and cancer.[2,6-8]

The link between chronic inflammation and cancer has long been suspected since Rudolf Virchow discovered immune cell infiltration in tumors in the mid-1800s.[9] It is estimated that 20% of human cancers are linked to chronic inflammation and persistent infection. Examples of such association include *Helicobacter pylori* infection with gastric cancer, HBV and HCV infections with hepatocellular carcinoma (HCC), and chronic inflammatory bowel diseases (IBD) with colorectal cancer (CRC).[10-12] Yet the majority of cancers that are not associated with preceding inflammation contain inflammatory infiltrates. The etiology of such "tumor-elicited inflammation," and its role in cancer development, had remained largely elusive.

We recently reported a novel mechanism for the induction of tumor-elicited inflammation. Using a mouse model of sporadic CRC, we observed a striking loss of barrier function in transformed epithelial cells of colonic adenomas.[13] Loss of epithelial barrier function results in infiltration of bacterial products that activate tumor-associated macrophages to induce the production of inflammatory cytokines including IL-23 and promote tumor growth and progression. Immune and myeloid cells in the tumor stoma that produce IL-17, IL-6, IL-22, and IL-23 play key roles in this process (**Fig. 1**).[13]

Mucosal layers and epithelial junctional structures serve as strong barriers that prevent translocation of bacteria and their products into intestinal tissue. Goblet cells produce mucus and secrete it into the lumen of intestinal tract. Highly glycosylated MUC2 protein forms a net-like polymer that spreads into layers above epithelial lining, and prevents close contact between the epithelial cells

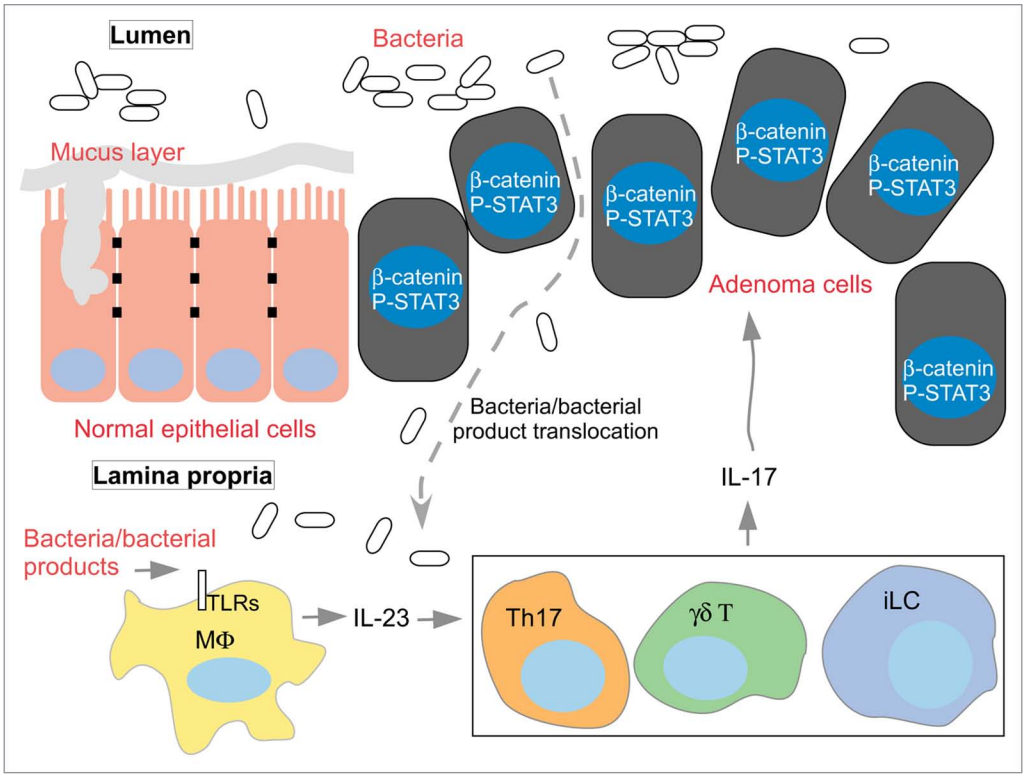


**Figure 1.** IL-23/IL-17 axis in tumor-elicited inflammation induced by barrier loss. Normal intestinal epithelium is covered by a mucus layer produced by goblet cells. Enterocytes also form tight junctions that control paracellular translocation of ions and molecules, and prevent translocation of commensal bacteria and their products into the lamina propria. Loss of *Apc* and activation of β-catenin induce adenoma formation in the intestine. Adenoma cells fail to produce mucus and form an effective intercellular junctional structure. As a consequence, gut bacteria and/or their products translocate into tumor stroma and activate tumor associated macrophages to produce IL-23, which in turn signals to Th17 cells and other IL-17 producing cells. IL-17 signals on adenoma cells and activates STAT3 indirectly to promote their proliferation.

and commensal bacteria.[14] Mice lacking MUC2 develop spontaneous colitis and colitis-associated CRC (CAC).[15,16] Ablation of MUC2 in $Apc^{Min}$ mice resulted in significantly increased intestinal tumorigenesis, especially in the colon.[17] Mice lacking the enzyme for mucin glycosylation also showed increased colonic permeability, and are highly susceptible to induction of colitis and CAC.[18] These data indicate that the mucus layer protects the host from colitis and CAC by keeping commensal microflora at a distance from the intestinal epithelium. Using a model of spontaneous CRC, we found that colonic adenomas are devoid of goblet cells that produce and secrete mucus.[13] MUC2 mRNA levels are significantly downregulated in both mouse and human colorectal adenomas, indicating defective mucus production and/or goblet cell differentiation already in early tumorigenesis.[13] The relationship between the mucus layer and CRC therefore becomes significant already at early stages of CRC development. Loss of mucin layer either before or early during CRC development serves as a driver of colonic tumorigenesis by augmenting tumor-promoting inflammation.

The intestinal epithelial barrier also depends on tight junction and adhesion junction proteins. These junctional structures define the apical and baso-lateral surface of polarized epithelial cells. Junctional structures also control paracellular traffic of ions and molecules, and prevent invasion of the epithelium by microorganisms.[19] Dysregulated junctional function and translocation of microbes is seen in multiple human diseases, including IBD, HIV infection, and alcohol abuse.[6] Ablation of JAM-A, a component of tight junction complex, resulted in enhanced colonic permeability and inflammation in mice.[20] Altered expression of tight junction proteins was also reported in human colorectal cancer tissues.[21] The change in junctional proteins was mostly speculated to be important in malignant progression of cancers. We found that the expression and localization of multiple junctional proteins, including claudins and junction–adhesion molecules (JAMs), are dysregulated in colonic adenomas compared with adjacent normal colon tissue.[13] Importantly, loss of barrier proteins was also observed in early human adenomas and in acutely induced aberrant foci in the mouse colon, suggesting that barrier defect is an early event during colon tumorigenesis.[13] Loss of the APC tumor suppressor gene and activation of β-catenin confers a proliferative state on enterocytes and blocks their differentiation.[22] Loss of barrier function may therefore come as a consequence of β-catenin activation and/or APC loss, in part due to loss of membrane localized, intact β-catenin, which controls cytoskeleton dynamics.[23]

With the loss of barrier function at the adenoma surface during early CRC

development, bacteria and/or their products come into close contact with transformed enterocytes and breach into the tumor stoma. Indeed, when injected into clamped mouse colon, fluorescent-labeled LPS was detected within tumors but not within adjacent normal tissue, and co-localized with the macrophage marker F4/80.[13] By using fluorescent in situ hybridization against bacterial 16S RNA, we also detected presence of bacteria in colorectal tumors and close to tumor epithelial cells of mouse and human early adenomas.[13] Infiltration of bacterial products serves as a driving force for tumor-elicited inflammation. The invading bacteria or bacterial products activate toll-like receptor (TLR)/MyD88 signaling in tumor associated macrophages, leading to the production of IL-23.

IL-23 belongs to the IL-12 family of heterodimeric cytokines that includes IL-12, IL-23, IL-27, and IL-35.[24,25] IL-23 is composed of a unique p19 subunit, and a p40 subunit which it shares with IL-12.[24-26] IL-23 is a major cytokine that promotes inflammation in a variety of autoimmune diseases. Ablation of the p19 subunit of IL-23 resulted in diminished experimental autoimmune encephalomyelitis (EAE) induction, similar to the effect of IL-12p40 deletion.[27] IL-23 was also found critical for other autoimmune diseases including rheumatoid arthritis,[28] psoriasis,[29] and IBD.[30-32] IL-23 is upregulated in multiple human cancers and ablation of the *Il23p19* gene resulted in reduced tumorigenesis in a mouse model of skin cancer.[33]

In colonic adenomas, IL-23 signals to hematopoietic cells and upregulates the expression of other cytokines including IL-6, IL-17, and IL-22. By doing so IL-23 indirectly activates STAT3 in tumor cells and promotes tumor growth.[13] It is noteworthy that in human patients with stage I/II colorectal cancer, a high "Th17 signature" confers drastically reduced disease-free survival after resection of primary tumors.[34] Screening of early CRC cases for IL-17 expression and adjuvant treatment of patients showing a high "Th17-signature" with IL-23 or IL-17 antagonists may prove beneficial in extending disease-free survival in early stage CRC.

Over 1000 species of mostly unculturable bacteria, at a sum total of 100 trillion, reside in the human colon and form a normally symbiotic relationship with their host. However, a dysregulated relationship between the host and flora bacteria also causes multiple human diseases, including infections, obesity, IBD, and CRC.[1,3,35] Translocation of bacteria into mesenteric lymph nodes signals poor prognosis in human CRC patients.[36] Entertoxigenic *Bacteroides fragilis* (ETBF) causes inflammatory diarrhea in some humans. Colonization of the mouse intestine with ETBF triggered colitis and strongly increased tumorigenesis in the $APC^{Min}$ model of CRC, with a robust IL-17 response and STAT3 activation in mouse colon.[37] ETBF also promotes colonic tumorigenesis by inducing the production of reactive oxygen species and DNA damage.[38] Infection of $APC^{Min}$ mice with *Citrobacter rodentium* also resulted in elevated tumor formation in the colon.[39] In a model of CAC driven by loss of IL-10, chronic inflammation disturbs microbiota composition and enriches the percentage of *E. coli* in mice reconstituted from a germ-free facility.[40] Polyketide synthase (pks) genotoxic island from *E. coli* is more prevalent in patients with IBD and CRC, and promotes colonic tumorigenesis and malignant progression in mice.[40] On the contrary, delivery of *Lactobacillus acidophilus* that is deficient in the production of lipoteichoic acid (LTA) protected mice against colitis, and caused regression of colonic adenomas.[41] LTA is a component of the cell wall of several gram-positive bacteria and is recognized by TLR2 to stimulate cytokine production by dendritic cells and possibly other immune and myeloid cells.[42] Use of LTA-deficient *L. acidophilus* as probiotics ameliorated detrimental inflammation in mice and protected them from colonic tumorigenesis.[41] Such an effect remains to be demonstrated in humans.

The discovery of barrier breach during adenoma formation as a driving force behind "tumor-elicited inflammation" and cancer progression adds to our current knowledge of the involvement of bacteria in CRC. In this case the otherwise "benign" bacteria in our microbiota can turn into an oncogenic factor due to altered host defense mechanism, suggesting the uniqueness of the mucosal environment in prevention of cancer development. Similar mechanism may exist in cancers of other bacteria-rich epithelial surfaces, including lung, skin, and other sections of the gastrointestinal tract.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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